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13. ABSTRACT (Maximum 200  <p><u>HUMAN STUDIES:</u> The effects of estrogen supplementation (ES) on heat acclimation was studied in 14 pre-menopausal females (18-35 yrs old) randomly assigned to either ES or placebo (P) groups after being matched for VO<sub>2</sub> max, percent body fat, and body weight/surface area ratio. Four days after the onset of menstruation they performed 2-h bouts of treadmill exercise (35-45% VO<sub>2</sub> max) daily in the heat (45°C, 20% RH) until acclimated. On day 2 of the menstrual cycle, subjects ingested either β-estradiol tablets (6 mg/day) or placebo tablets, for 7 d. Based on thermal and circulatory measures, HSP70 synthesis, and days to achieve acclimation, we conclude that ES, as performed in this study, had no effect on heat acclimation. <u>ANIMAL STUDIES:</u> In the animal study, rats received daily subcutaneous injections of estradiol (10 ug/100 ml/g b.w.). One group underwent a daily exertional heating protocol (trained) and a second group served as sham controls (untrained). Within each group, 3 subgroups were utilized to assess the time course of potential alterations: a) 4-day, b) 8-day, or c) 12-day. On the final day of a protocol, rats underwent a heat tolerance test consisting of treadmill exercise at 21.5 m/min at 35°C until colonic temperature (T<sub>c</sub>) reached 40.4°C. In general, rats in the trained group had lower body weights, reduced resting T<sub>c</sub>'s, attenuated heating rates, and increased run times to 40.4°C (P&lt;0.05) than their untrained counterparts. These results were primarily manifested in rats trained for 8 or 12 days compared with the 4-day treatment group. These studies demonstrate that the combination of exertional heat exposure and estradiol treatment, when compared to estradiol supplementation alone, enhances thermotolerance in rats exercising at a high ambient temperature.</p>				
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Carl T. Smith  
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## I. Human Studies

### A. Effect of estrogen supplementation on heat acclimation in pre-menopausal females

1. **Introduction.** Heat acclimation can usually be achieved by performing mild to moderate exercise (30-55%  $\text{VO}_2$  max, 2-4 h·day<sup>-1</sup>) in a hot environment for 5-12 days. The state of acclimation is characterized by decreased core and skin temperature, decreased heat storage, increased sweating rate, and a lower sweating and vasodilation threshold (24, 25, 35, 37). The acclimation process is similar in women and men of comparable aerobic fitness (1), and is independent of the menstrual cycle (20).

Reducing the duration of time needed to achieve heat acclimation would be beneficial under situations where exercise in the heat was necessary after limited notice. Recent evidence suggests that estradiol supplementation (ES) should accelerate the heat acclimation process. In post-menopausal women ES improves thermoregulatory responses during exercise in the heat (33). Furthermore, recent animal studies demonstrate that estradiol therapy increases evaporative water loss at all levels of core body temperature, reduces the core body temperature threshold for onset of saliva spreading (2), and elevates heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90) concentrations in the ventromedial hypothalamus in ovariectomized female rats (19). No study to date has examined the time required to develop heat acclimation in menstruating women receiving estrogen supplementation or in post-menopausal women undergoing estrogen replacement therapy.

The most temperature sensitive and highly conserved of the heat shock proteins is HSP70. At the cellular level, all organisms respond to stress by synthesizing HSP's which are considered the central component of acquired heat tolerance (16, 17, 18). Ryan et al. (27) demonstrated that HSP70 is produced in humans exercising in the heat, and thus HSP70 maybe a useful biomarker to investigate the adaptation to thermal stress.

Wright (36) reported that the maximal core temperature of mice can be increased by heat acclimation; however, the mechanisms for this observation were not examined. Highly trained human marathon runners can tolerate core temperatures between 40°C - 42°C without signs of heat illness (22, 37). Two species of antelope, Grant's gaelle and oryx, can survive the rigors of desert life, in part, by allowing their core temperature to rise above over 46°C (34). These observations suggest that tissue tolerance to high temperature may be developed in organisms repeatedly exposed to heat stress.

Thus, the purpose of this study was to determine: a) whether ES will reduce the time required to achieve heat acclimation in pre-menopausal females during the early follicular phase of the menstrual cycle, and b) whether levels of leukocyte HSP70 can serve as a biomarker of heat acclimation in these individuals. Because ES may increase HSP70 and improves thermoregulation in post-menopausal women, we hypothesized that ES will reduce the time required to achieve heat acclimation and that the level of leukocyte HSP70 will serve as a convenient and rapidly assayed biomarker of heat acclimation. This study therefore allows investigation of cellular mechanisms in the heat acclimation process.

### 2. Methods.

*Subjects.* Fourteen females, aged 18-35 yrs, volunteered to participate in the study. Subjects were sedentary, did not smoke, and did not use oral contraceptives for at least 2 months prior to the experiment. All subjects exhibited a normal menstrual cycle. Each volunteer was informed of testing procedures, written consent was obtained, and each received a physical examination. All experimental procedures were approved in advance by the Human Subjects Committee at the University of Iowa.

Experiments were conducted between February and May 15 to ensure that subjects were not heat acclimatized prior to the study. Subjects recorded their basal rectal temperature for at least one cycle prior to the experiment. There was no significant difference between groups for maximal oxygen consumption ( $\text{VO}_2$ max), age, percent body fat, and body weight/surface area ratio (Table 1). Serum estradiol and progesterone concentrations were obtained on the first day of menstruation to confirm the follicular phase of the period (Table 2).

*Estimation of maximal oxygen uptake and submaximal oxygen uptake.* Before the experimental trials, each subject's  $\text{VO}_2$ max was determined using a graded incremental treadmill test to volitional fatigue.

Oxygen uptake ( $\text{VO}_2$ ) and carbon dioxide production were analyzed continuously (Q-Plex I Metabolic System, Quinton Instruments Co., Seattle, WA), and heart rates were measured every min.  $\text{VO}_{2\text{max}}$  was defined as achieving of at least two of the following three criteria: a plateau or decrease in  $\text{VO}_2$  despite an increase in workload, a respiratory exchange ratio greater than or equal to 1.1, and/or attainment of at least 90% of predicted maximum heart rate (5). The workload required to elicit 35-45%  $\text{VO}_{2\text{max}}$  was calculated from submaximal  $\text{VO}_2$  values.

*Study design.* Subjects were studied during the follicular phase of the menstrual cycle to minimize possible effects of endogenous progesterone and to maximize differences in estradiol levels between ES and control subjects. There were two experimental time periods. One group ( $n=6$ ) started experiments at 6:00 a.m., whereas another began at 3:30 p.m. Each subject exercised at the same time of the day throughout the study. Subjects who exercised in the same group were paired based on  $\text{VO}_{2\text{max}}$ , percent body fat, and body weight to surface area ratio. Body surface area was determined by the Dubois formula (6). Using a double-blind design, subjects were randomly assigned to either the ES ( $n=7$ ) or placebo (P;  $n=7$ ) groups. To minimize training effects, subjects walked on a treadmill (35-45%  $\text{VO}_{2\text{max}}$  for 2 h) in a cool environment (22-24°C, 20% RH) for 5 days beginning 3 days before the expected start of their menstrual cycle. On days 2-8 of the menstrual cycle either estradiol (Estrace®, 2 mg-tablet<sup>-1</sup>, Bristol-Meyers Squibb Co., Princeton, NJ) or placebo tablets were ingested (3 tablets-day<sup>-1</sup>). On day 4 of their menstrual cycle, subjects began heat acclimation in a heat chamber (45° C, 20% RH) by walking at 35-45%  $\text{VO}_{2\text{max}}$  for 2 h. Heat acclimation was identified when rectal temperature ( $T_r$ ) stabilized ( $\leq 0.1^\circ\text{C}$ ) during the last 30 min of the 2 h walk in the heat. To ensure subject safety, exercise was terminated when: a) rectal temperature ( $T_r$ ) exceeded 39.5°C; b) symptoms of impending syncope, nausea, dizziness, or headache occurred; c) heart rate (HR) exceeded 95% of the predicted maximum HR; or d) volitional fatigue.

Blood samples (20 ml) were obtained on the first day of the menstrual cycle, immediately before and after exercise on the last day in a cool environment and on the first, fifth, and final day in a hot environment. These were analyzed for leukocyte HSP70, serum estradiol and serum progesterone.

*Experimental protocol.* Upon arriving to the lab, subjects ingested 6 ml·kg<sup>-1</sup> of water to ensure hydration. Subjects then stood for 20 min for plasma volume equilibration and a blood sample (20 ml) was taken. A nude body weight was obtained after voiding, a rectal thermocouple probe (ESO-1, Physitemp Instruments, Inc., Clifton, NJ) interfaced to a thermocouple digital recorder (Model HH201, Omega Engineering, Inc., Stamford, CT) was inserted 10 cm beyond the anal sphincter, and a heart rate monitor (Polar Vantage XL, Polar USA, Stamford, CT) was attached. Subjects walked 2 h in the cool environment ingesting 2.5 ml·kg<sup>-1</sup> of water every 20 min. Immediately after exercise, a nude body weight, and blood and urine samples were obtained. Exercise in the heat began on day 4 of the menstrual cycle. In these experiments, skin temperature was monitored and water intake was increased to 5 ml·kg<sup>-1</sup> every 20 min.

*Measurements.*  $T_r$ , mean skin temperature (MST), perceived exertion (PE), thermal sensation (TS), and HR were measured on day 1 and day 5 in the cool environment (22-25°C), and on each day of heat acclimation.  $T_r$  and HR were measured every 5 min, TS and PE every 10 min, and MST every 2.5 min.  $\text{VO}_2$  was measured 5-10 min into experiments on the first and last day in the cool environment, and on the first, third, fifth and last day in the hot environment.

Percent body fat was estimated from the sum of triceps, suprailiac, and thigh skinfolds (21). Skin temperatures were collected each min by an on-line computer system (IBM AT Personal Computer, IBM Corp., Armonk, NY). Mean skin temperature was reported as the weighted sum of 4 sites (chest 0.3 + upper arm 0.3 + thigh 0.2 + lower leg 0.2) (23). Measurements of PE and TS were obtained as described by Borg (3) and Gage (10). Sweat rate (SR) was determined from the change in nude body weight pre- to post-exercise corrected for water intake and urine output.

*Blood analysis.* Serum estradiol and progesterone were measured by <sup>125</sup>I radioimmunoassays. Leukocyte HSP70 was determined as described previously (27). Briefly, 5 ml of Histopaque (Sigma Chem. Co., St. Louis, MO) was added to ~10 ml blood and centrifuged at 3000 rpm for 25 min to isolate the leukocytes. The leukocytes were then washed (gentle aspiration and centrifugation at 1,500 rpm for 5-7 min) twice with phosphate buffer solution (PBS). The final leukocyte pellet was suspended in 0.25 ml PBS, and stored at -70°C until analysis. This isolation procedure was initiated immediately on attainment of a blood sample. HSP70 analysis was performed using western blot analysis.

*Statistical analysis.* One and two-factor analysis of variance (ANOVA) with repeated measures were utilized to determine if significant differences existed between groups and across time. The Scheffe

post-hoc test was employed to determine the location of any significant differences ( $P < 0.05$ ). Values presented are mean  $\pm$  SE.

**3. Results.** Serum estrogen concentration was significantly elevated after 3 and 7 days of ES compared to P. Serum progesterone was not significantly different from day 1 to 7 and was similar between groups (Table 2).

The number of days required to achieve heat acclimation was not reduced by ES ( $6 \pm 0.5$ ,  $6 \pm 0.5$  days in ES and P groups, respectively).  $T_r$  on the first and last days in the heat was also similar between groups (Fig. 1C). These results indicate that 7 days of ES had no effect on  $T_r$  during rest or exercise in the heat in pre-menopausal women during the follicular phase of the menstrual cycle.

Synthesis of HSP70 also did not significantly differ between ES and P on any day of blood measurement. HSP70 was elevated on the last day of ES before exercise, however, due to high variability among subjects, there were no significant differences between or within groups (Fig. 3).

SR on day 1 and the final day of heat acclimation was not significantly different between the ES and P groups and no significant difference existed between day 1 and the final day of heat acclimation (Fig. 4). HR, MST,  $T_r$ , TS, and PE (Fig. 1A, 1B, 1C and Fig. 2A, 2B) were significantly lowered on the last day of heat acclimation.  $VO_2$  was similar on days 1, 3, 5, and the final day in the heat for each group and no significant difference was found between groups on any day (Table 3).

**4. Discussion.** Results from this study show that the time required to achieve heat acclimation was not reduced by 7 days of ES in pre-menopausal women during the follicular phase of their menstrual cycle.  $T_r$  was not altered at rest or during 2 h exercise in the heat and the synthesis of HSP70 was not induced by ES. Heat acclimation significantly reduced HR, MST, TS and PE, but these physiological responses were not altered by ES. SR and  $VO_2$  were not altered by heat acclimation or ES.

HR was significantly reduced on the final day of acclimation following 60 min of exercise in the heat (Fig. 1A). Several mechanisms have been proposed for lowering HR over the course of heat acclimation: 1) increased plasma volume (14, 31, 35); 2) redistribution of cardiac output away from cutaneous vascular beds (32); and 3) decreased thermal drive associated with the fall in core and skin temperatures (26). The time course of the decrease in HR is roughly similar to that of the increase in plasma volume, and the two changes are significantly correlated (31). Furthermore, Senay (29) has hypothesized that even after plasma volume at rest returns toward normal, plasma volume during exercise in the heat is still larger after acclimation, because of an enhanced hemodilution response during exercise in the heat. Since HR at a given core temperature is the same or higher after acclimation than before (8, 9), lower body temperatures produce lower HR. However, the evidence available is not sufficient to establish a specific mechanism(s) to explain the cardiovascular improvements resulting from heat acclimation (35).

Several studies have demonstrated that SR significantly increases with heat acclimation (7, 14, 15, 30, 37). An increase in SR, however, is not a prerequisite for heat acclimation. In this study SR was not significantly elevated ( $p > 0.05$ ) after heat acclimation in either group, and no significant difference occurred between groups. Sawka et al. (28) also did not find an increased SR in pre-menopausal women following 10 days of heat acclimation to alternate hot-dry ( $49^\circ\text{C}$ , 20% RH)/hot-wet ( $35^\circ\text{C}$ , 79% RH) environments. Horstman and Christensen (11) did not find increases in SR after 6 days of heat acclimation ( $45^\circ\text{C}$  dry-bulb,  $23^\circ\text{C}$  wet-bulb environments) in pre-menopausal women, however, they did observe a significant increase after 11 days. Unfortunately, menstrual cycle phase was not indicated in either study. Avellini et al. (1) demonstrated that after 10 days of heat acclimation in a  $36^\circ\text{C}$  dry-bulb/ $32^\circ\text{C}$  wet-bulb environment, SR was similar at 30, 90, and 120 min of exercise-heat exposure. Furthermore, SR during pre- and post-ovulation was similar before and after heat acclimation. Maher (13), and Robinson (25) did not find increased SR in men after heat acclimation. These experiments were conducted in a dry heat environment. Increases in SR after heat acclimation may be related to high ambient water vapor pressures. Collins et al. (4) reported that in dry heat nearly all of the sweat produced is evaporated and acclimation increases SR only enough to reduce heat storage and to compensate for the alteration in sensible heat exchange associated with lowered skin temperature. Another possibility is that sweating is more evenly distributed after heat acclimation leading to enhanced evaporative cooling. These data may explain significantly lower MST after heat acclimation without increases in SR. Fortney and Senay (7) reported an increase in SR did not occur until after 7 days of acclimation for most subjects. In the present study, our subjects reached heat acclimation in less than 7 days.

MST has a strong relationship with the sensation of comfort, and the consequent use in models predicting comfort (12). Fortney and Senay (7) suggested that a reduction in peripheral blood flow may explain the significantly lowered MST after heat acclimation, since only part of which could be accounted for an increase in SR. Roberts et al. (24) have also reported lowered skin conductances following heat acclimation. Since our subjects' MST during exercise in the heat were higher before, than after acclimatization, it is obvious that heat exchange was more favorable following heat acclimation.

TS and PE were improved after heat acclimation (Fig. 2A, 2B). This improvement is attributed to a lower core temperature and HR (9). It is also possible that the lowered skin temperature achieved with acclimation (12) allowed a greater thermal gradient from core to surface, reducing demands for cutaneous blood flow.

Synthesis of HSP70 was not induced by 7 days of ES or by heat acclimation. Although, there was a trend for HSP70 synthesis to rise on the last day of ES, there were no significant differences between groups or between blood samples within a group. Ryan et al. (27) demonstrated that synthesis of HSP70 in humans was induced when the core temperature reached 40.2 to 40.7°C. In our study, the highest  $T_r$  was 39.3°C. These data suggest that 5 to 8 days of exercise in the heat is not sufficient to induce HSP70 when  $T_r$ 's are below 39.3°C. Olazabal et al. (19) reported that estradiol significantly induced HSP70 synthesis in the ventromedial hypothalamus of females rats after 12 h of hormone treatment. In the present study, human leukocytes did not increase synthesis of HSP70 with increased plasma estrogen concentrations suggesting that tissue (16) and/or species differences may exist.

We conclude that 7 days of ES had no effect on the time required to reach heat acclimation in premenopausal women during the follicular phase of the menstrual cycle.  $T_r$ , HR, MST, SR, TS, and PE responses were similar throughout the heat acclimation protocol between the two groups. Furthermore, synthesis of HSP70 was not induced by ES or by the heat acclimation process.

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## 6. Tables 1, 2, 3

**Table 1. Subject characteristics.**

Group	Height (cm)	Weight (kg)	Age (y)	VO <sub>2</sub> max (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	% Body Fat	BW/SA
Estrogen	168.2± 3.3	62.5 ± 4.0	24.3±1.4	39.5 ± 1.3	24.1 ± 2.6	36.9 ± 1
Placebo	169.2±2.1	64.1 ± 3.3	25.1±1.9	40.8±1.9	24.8 ± 2.2	36.9 ± .9

Values are means ± SE.

**Table 2. Plasma estradiol and progesterone concentrations on the day before, and on days 3 and 5 of estrogen supplementation.**

Group	Estradiol (pg·ml <sup>-1</sup> )	Progesterone (ng·ml <sup>-1</sup> )
<u>Estrogen</u>		
Pre	30.9 ± 6.8	0.8 ± 0.1
Day 3	870.2 ± 148.3 *	0.6 ± 0.1
Day 7	1062.7 ± 168.1*	0.7 ± 0.1
<u>Placebo</u>		
Pre	31.7 ± 4.9	2.2 ± 1.6
Day 3	61.2 ± 21.6	0.6 ± 0.2
Day 7	59.0 ± 10.7	0.6 ± 0.1

\* Significantly different from placebo groups (P < 0.001). Values are means ± SE.

**Table 3. Oxygen uptake during treadmill walking on day 1, 3, 5, and the final day in the heat.**

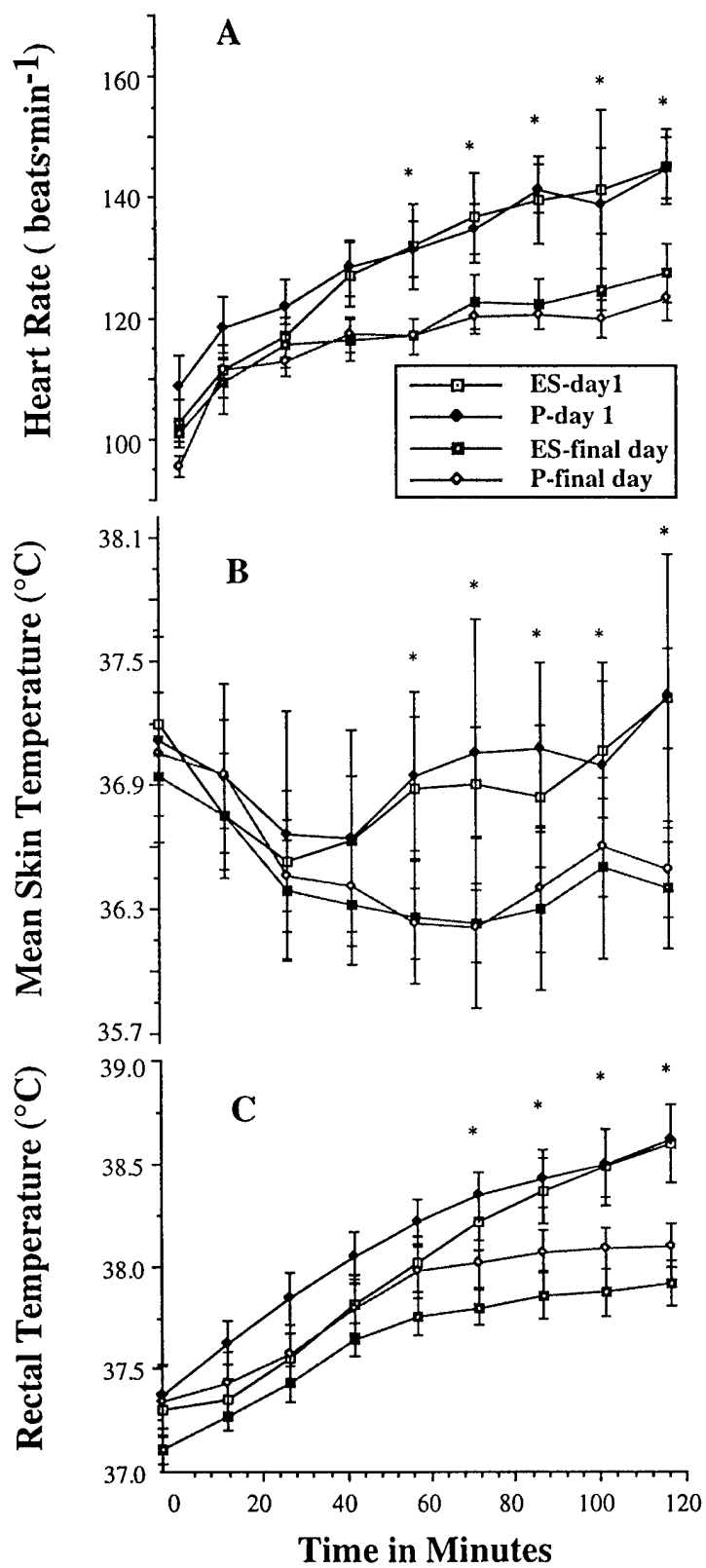
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )				
Condition	Day 1	Day 3	Day 5	Final Day
Estrogen	41.2±2.48	39.3±2.28	41.8±2.55	39.48±2.31
Placebo	38.1±1.22	39.2±3.74	42.0±3.36	42±5.29

Values are means ± SE.

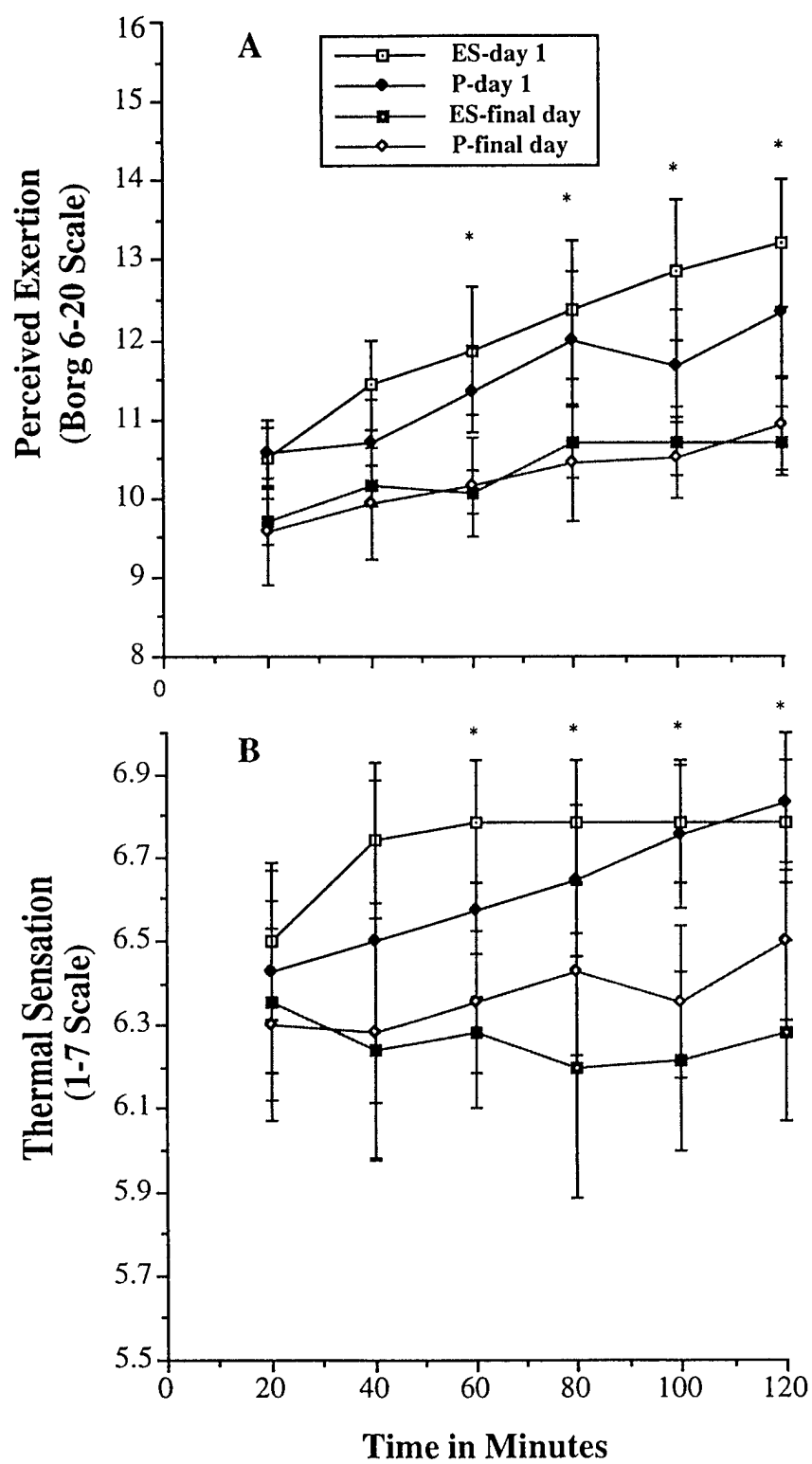
## 7. Figure Legends

- Fig. 1. Heart rate, mean skin temperature, and rectal temperature on day 1 and the final day of heat acclimation. (ES: estrogen supplementation, group; P: placebo group). Values are  $\pm$  SE.
- Fig. 2. Perceived exertion, and thermal sensation on day 1 and the final day of heat acclimation.
- Fig. 3. Leukocyte HSP70 concentration. Data are expressed as a percentage of day 2 pre-exercise ES or P supplementation. Abbreviations: HSP70: heat shock protein 70; ES: estrogen supplementation group; P: placebo group; 1 pre Ex: day 1 in the heat before exercise; 1 post Ex: day 1 in the heat after exercise; 5 pre Ex: day 5 in the heat before exercise; 5 post Ex: day 5 in the heat after exercise; F pre Ex: final day in the heat before exercise; F post Ex: final day in the heat after exercise.
- Fig. 4. Sweat rate on day 1 and the final day of heat acclimation in each group.

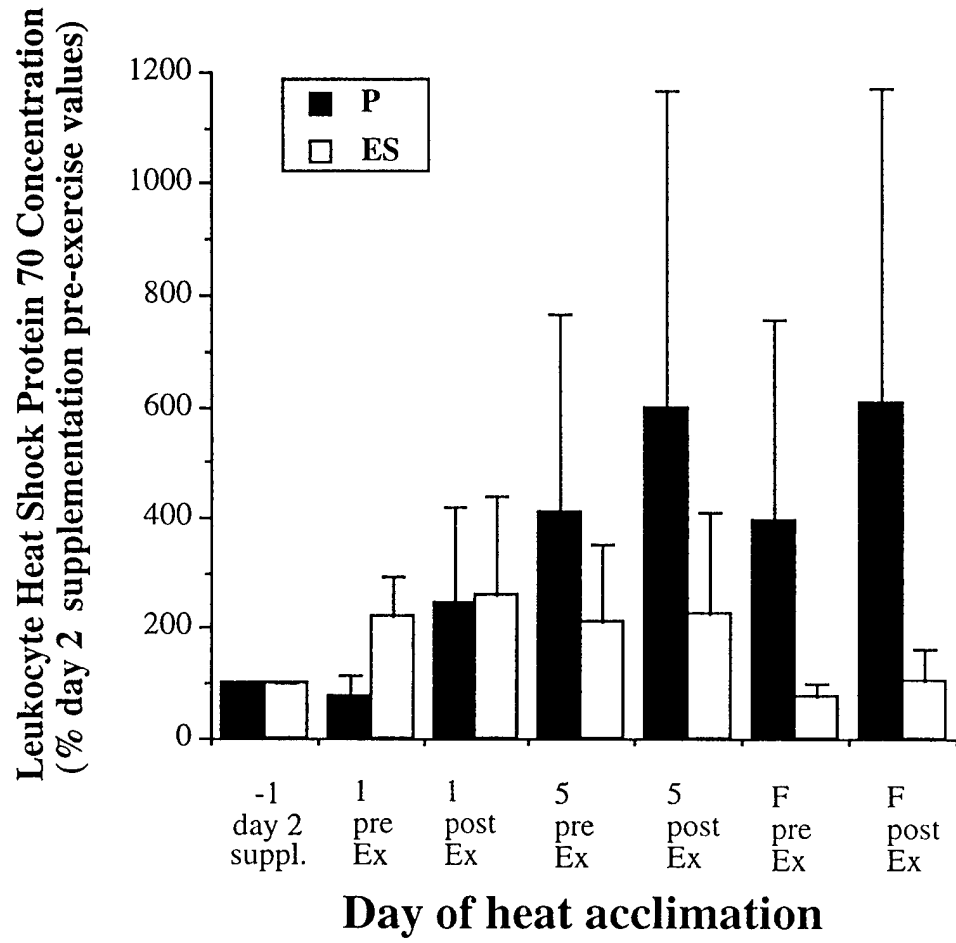
**Fig. 1**



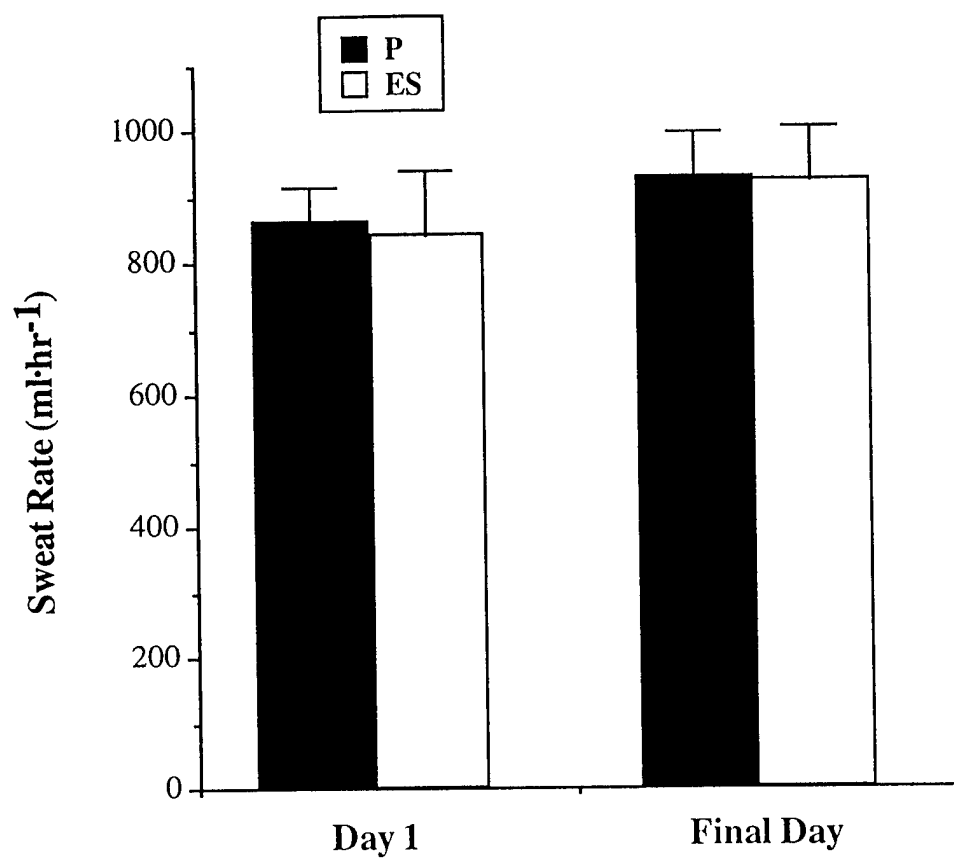
**Fig. 2**



**Fig. 3**



**Fig. 4**



## II. Animal Studies

### A. Effects of estradiol and training on thermotolerance during exercise and heat stress

*Since last year's animal report was prepared, we have conducted additional animal experiments. The results of these experiments are described below.*

1. **Introduction.** The purpose of these studies is to test the hypothesis that estrogen therapy in ovariectomized female rats is associated with enhanced thermotolerance to exercise in the heat. The unique contribution of these experiments lies in our methodology and approach to the problem. Hyperthermia is a physiological stress regularly experienced by a majority of our population. It is noninvasive and does not make use of exogenous pharmacological manipulation. Moreover, a variety of questions, including hormonal contributions to thermoregulation, exercise performance in the heat, and cellular stress responses may be addressed. The rat is a useful animal for studying the mechanisms of action of estrogen on thermoregulation in heat stress and can be utilized to define the upper limits of temperature exposure and the tissue specificity of the response. The information derived from these studies may in turn be an indication of therapeutic interventions for athletes competing in the heat.

#### 2. Methods.

**Animals.** Two groups of 8-week-old female Sprague-Dawley rats (Harlan, Madison, WI) were utilized to conduct these experiments: an untrained, estradiol-treated control group (250-300 g, n=22) and a trained, estradiol-treated group (200-250 g, n=23) (see Table 1). Training consisted of an exertional heating protocol performed on a daily basis for a designated number of days (described in detail below). Ovariectomized rats were used to precisely control for the effects of female reproductive hormones on the HSP response. Ovariectomized rats have no estrous cycle and no circulating plasma estrogen and progesterone, thereby enabling experimental manipulation of hormone concentrations. Ovariectomies were performed by the supplier prior to shipment. Rats were housed in group cages in a temperature-controlled animal facility with a 12:12-h light-dark cycle and were provided standard rat chow and water ad libitum. Experiments and animal care procedures were performed in accordance with institutional guidelines. All rats were familiarized with the testing environment and a colonic thermistor probe several times over the week preceding an experiment. Animal weights were recorded immediately before and after each experiment.

**Experimental Protocol.** Within each of the two groups, animals were randomly divided into three sub-groups, each with a separate protocol dependent upon the duration of injections: 1) A four-day treatment protocol, 2) An eight-day treatment protocol, and 3) A twelve-day treatment protocol. Throughout the treatment period, each rat was weighed at 8:00 am and the amount of estradiol to be injected was calculated. The estradiol-treated groups were given a pharmacological dose of 17 $\beta$ -estradiol 3-benzoate (Sigma Chemical, St. Louis, MO); 10 mg/0.1 ml of sesame oil/100 g body weight-1 was injected into the subcutaneous dorsal neck skinfold. Each injection was administered by one investigator at the same time of day to ensure consistency of hormone treatment throughout the protocol.

Three hours after the injections, a colonic thermistor probe was inserted 6 cm beyond the anal sphincter and into the colon (Tc) for measurement of internal body temperature. Pre-exercise Tc was recorded and then rats were placed on a treadmill and walked at a pace of 21.5 m/min at a 0% grade and timed until their temperature reached 40.4°C. Rats were then removed from the treadmill, weighed, and returned to their home cages.

On the final day of the study (either the fourth, eighth, or twelfth day), a heat tolerance test (HTT) was administered. An injection of the vehicle solution (0.1 ml sesame oil/100 g body weight dose) was given at 8:00 am and four hours later the rats were weighed, the colonic thermistor probe was inserted, and Tc was recorded. The rats were then placed on a treadmill at an ambient temperature of 35°C. The rats walked at 21.5 m/min at a 0% grade and the experimental protocol was terminated when the animals attained a Tc of 41.5°C. Upon completion of the exercise challenge, the rats were removed from the treadmill unit and placed into their home cage for four hours prior to collection of tissue samples. The rationale for choosing this time point was that previous data indicate that maximal HSP70 induction to estradiol occurs in this 4-12 hour post-injection period (2-4).

*Assays.* Following the four hour waiting period to allow adequate time for protein synthesis, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). It has been shown that this procedure will have no effect on HSP70 synthesis (1). Tissues (heart, lung, liver, intestine, kidney, skeletal muscle) were removed and frozen in liquid nitrogen. HSP70 assays will be performed by Dr. Moseley over the course of the next several months.

*Analysis.* There were 45 ovariectomized female rats separated into two estrogen treatment groups consisting of a heat/exercise-conditioned group and an unconditioned group. These groups were in turn divided into three sub-groups depending upon the duration of the treatment protocol. All results are presented as means  $\pm$  SE. Appropriate statistical analysis was performed to determine significant differences in animal weight (pre- vs. post-HTT), initial and final Tc values, heating rate, and heating rate as a function of body mass. Significant differences were determined with an analysis of variance for two factors design (Comparison between protocols within each treatment group and comparison between treatment groups within a given protocol) followed by Duncan's post hoc analysis. Significance was established at the  $P < 0.05$  level.

### 3. Results

#### Weight:

*Within groups:* The general trend was for body weight to decrease as the length of treatment increased (Table 2). In the untrained group (Group I), 12-day rats had a significantly lower body weight than either 4-day or 8-day rats. In the trained group (Group II), both 8-day and 12-day rats had reduced body weights compared to the 4-day rats.

*Between groups:* The trained group had a lower body weight at both the 8-day and 12-day time points than their untrained counterparts.

#### Pre-HTT Core Temperature (Tc):

*Within groups:* In general, resting Tc prior to a HTT was similar between days within both treatment groups (Table 2).

*Between groups:* In comparisons between groups, both the 8-day and 12-day resting Tc's were lower in the trained compared with the untrained group.

#### Run-Time During HTT:

*Within groups:* In the trained, estrogen-treated group, there was a large increase in run-time in the 12-day vs. the 4-day and 8-day groups. There was no consistent trend in the untrained group (Table 2).

*Between groups:* There was a general trend for the trained group to have significantly greater run times than their untrained counterparts.

#### Heating Rate:

*Within groups:* In the trained, estrogen-treated group, there was a marked reduction in heating rates for the 12-day vs. the 4-day and 8-day groups (Table 2). Similar to the run-time values, there was no consistent trend in the untrained group for heating rates.

*Between groups:* There was a general trend for the trained group to have significantly reduced heating rates than their untrained counterparts. Specifically, both the trained 4-day and 12-day rats had large decreases in heating rates compared to comparable untrained groups.

#### Heating Rate per kg Body Weight:

The heating rate per kg was calculated as the quotient of the change in Tc ( $^{\circ}\text{C}$ ) and the product of a rat's body weight (kg) and the length of time (in min) to raise Tc to  $41.5^{\circ}\text{C}$  during the HTT (Table 2).

*Within groups:* In the untrained, estrogen-treated group, the heating rate per kg body weight was variable. In the trained group, rats in the 12-day group had reduced heating rate per kg values compared with both 4-day and 8-day rats.

*Between groups:* There was a general trend for the trained group to have significantly lower heating rates per kg than their untrained counterparts. Both the trained 4-day and 12-day rats had large decreases in heating rates compared to their respective untrained groups.

4. **Summary.** The initial sets of experiments, which were described in detail in last year's report, provided evidence that administration of estradiol for eight and twelve days increases

thermotolerance in ovariectomized rats. In addition, the data were indicative of a time course of hormonal therapy necessary to enhance performance during an exercise and heat challenge in ovariectomized rats, and that estrogen's effects on thermotolerance reach a plateau within eight days of treatment. These results could be due to enhanced thermoregulatory responses during exercise in a warm environment. Furthermore, estradiol-induced plasma volume expansion could also contribute to improving thermoregulation. Both of these factors would play a role in elevating evaporative cooling and heat loss mechanisms. In addition, estradiol treatment may result in a decreased rate of tissue glycogen utilization secondary to an estradiol-mediated increase in the availability of lipid substrate during the exercise intervention in the heat.

In the most recent experiments, our data indicate that the combination of training and estradiol treatment significantly enhance thermotolerance in rats, as evidenced by a decreased resting T<sub>c</sub> and a reduced heating rate during a HTT, compared with estradiol treatment alone. In addition, the 8-day and 12-day treatment groups consistently demonstrated better acclimation than the 4-day treatment groups. During the final year of this project, we will complete these studies by assessing in greater detail the effects of estradiol supplementation and training on heat tolerance and work performance. We will then be able to compare a variety of treatment groups with appropriate control groups to more completely discern the impact of both exercise training in a warm environment and estrogen supplementation on the ability of an organism to cope with a exertional heat challenge. In addition, during the next year we will have assays completed involving tissue HSP levels in the various treatment groups, which should provide insight into the potential role of these stress proteins on heat tolerance.

## 5. References

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3. Olazabal, U.E., D.W. Pfaff, and C.V. Mores. Estrogenic regulation of heat shock protein 90 kDa in the rat ventromedial hypothalamus and uterus. Molecular and Cellular Endocrinology. 84:175-183, 1992.
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## 6. Tables 1 & 2

**Table 1.**

	Conditioned Heat Treatment	Hormone during Pre-HTT period	Hormone during HTT
<b>Group I</b>	No	Estradial	Vehicle
<b>Group II</b>	Yes	Estradial	Vehicle

HTT = heat tolerance test

**Table 2.**

	Weight (gm)	Initial Core Temp.(°C)	Time to 41.5 (min)	Heating Rate (Δ°C/min)	Heat Rate/kg (Δ°C/kgmin)
<b>GROUP I</b>					
4 day (N=7)	226±1.5	37.73±0.101	40.1±8.26	0.111±0.0142	0.494±0.0619
8 day (n=7)	223±2.6	37.43±0.206	61.7±9.04	0.0771±0.0154	0.333±0.0601*
12 day (n=8)	217±2.0*#	38.30±0.159*#	31.5±5.72#	0.124±0.0194*	0.562±0.0851#
<b>GROUP II</b>					
4 day (n=7)	223±4.1	37.58±0.141	84.3±14.29†	0.054±0.0092†	0.245±0.0407†
8 day (n=7)	215±2.8*†	36.92±0.071*†	85.5±13.61	0.0617±0.0108	0.293±0.0518
12 day (n=8)	211±2.4*	37.75±0.091#†	115.2±4.80*#†	0.0325±0.0017*#†	0.155±0.00877*#†

\*p<0.05 vs. 4-day

#p<0.05 vs. 8-day

†p<0.05 Group I vs. Group II